# HSB Project 4

## Studies to Identify Environmental Cardiotoxins and Susceptibilities to Cardiotoxicity

**Project Leaders**
June K. Dunnick, Ph.D.
Scott S. Auerbach, Ph.D.

### *Background and Rationale*

Heart disease is the leading cause of mortality (National Center for Health Statistics 2005a) and health care costs (National Center for Health Statistics 2005b) in the United States. A combination of genetic and environmental factors (e.g. diet, exercise, cardiotoxins) determines ones risk of developing cardiac disease. Often the outcome of cardiotoxin exposure is influenced by an underlying susceptibility (e.g. genetic or sub-clinical disease). Current approaches to cardiac safety testing are still evolving and are limited in their ability to detect some forms of cardiotoxicity (Kettenhofen and Bohlen, 2008). Nowhere is this better illustrated than with the unanticipated clinical safety problems associated with the use of COX2 inhibitors (Patrono and Rocca, 2009).

One component of the NTP mission is to develop better testing methods. Along these lines, NTP has created a program to identify more effective methods for evaluating cardiotoxicity. Currently there are three components of this program: (1) identification of sensitive and specific biomarkers of cardiotoxicity (Dunnick *et al.*, 2007a); (2) identification of more sensitive models for cardiotoxicity testing, and; (3) discovery of genetic determinants of chemical-induced cardiotoxicity (CICT). Current studies being carried out by the program are centered on two cardiotoxins nominated to NTP for testing, bis(2-chloroethoxy) methane (CEM), and ephedrine/caffeine (E/C).

These chemicals are mechanistically distinct with respect to their cardiotoxic effects (Dunnick *et al.*, 2007b; Dunnick *et al.*, 2004). Although this is an integrated program only the genetic studies are described in this research concept. In short, we propose quantifying the acute cardiotoxicity phenotype of 34 inbred strains (listed below) following treatment with CEM and E/C using a serum biomarker(s) indicative of cardiac damage. We will then use this quantitative trait to perform in silico (haplotype association) mapping of genetic loci that confer differential susceptibility to cardiotoxin challenge.

Overall, this program fulfills the NTP Roadmap goals of transforming toxicology from an observational to a predictive science, developing translational biomarkers, and providing a better understanding of the pathogenesis of environmentally induced disease**.**

### *Key Issues*

It will be essential to properly design the proposed studies with sufficient power in order to identify genetic variation that modifies CICT. Others have been successful in identifying a genetic risk factor for acetaminophen-induced hepatotoxicity using a panel

of 36 inbred mouse strains (Harrill *et al.*, 2009). Our initial estimates indicate that use of 34 strains as proposed here will provide 60 to 80% power to detect a genetic association with CICT.

It is likely that the 34 mouse strain, in silico (haplotype association) mapping approach will lead to the identification of a large number of QTL, each of which will contain a number of genes. For this reason, additional data will be critical for determining which are likely to contain modifiers of CICT and which genes within the loci confer the effect. Along these lines we propose genetical genomic studies from cardiac tissue in the 34 strains of mice used to map cardiotoxicity QTL. Overlap between gene expression QTL and cardiotoxicity-modifier loci will be considered a strong indication of a genuine modifier locus. Further filtering of modifier loci will be performed using bioinformatics approaches which will consider the biological plausibility of candidate genes in the selected loci. Such approaches will include, but will not be limited to co-expression with cardiac disease genes (Jupiter and VanBuren, 2008), association with signaling networks (diseaseome (Goh *et al.,* 2007)) integral to cardiac function and variation in the protein coding or regulatory sequence of candidate genes.

***Approach and Specific Aims***

We plan to expose 34 strains of mice to 2 mechanistically distinct cardiotoxins (CEM and E/C) and quantify the extent of cardiotoxicity using serum biomarkers. Cardiotoxicity will be treated as a quantitative trait and used to map genetic loci in silico that confer differential sensitivity to cardiac challenge. Candidate genes will be identified in the QTL using genetical genomic, bioinformatic and systems biology approaches as reviewed elsewhere (Zhu and Zhao, 2007).

**Specific Aim 1: Determine the baseline cardiac phenotype in 34 inbred mouse strains using heart gene expression analysis, histopathology, electrophysiology, and serum biomarkers.** The gene expression studies will be used to map genetic determinants of differential expression. Other endpoints will be used to identify any sub-clinical cardiac disease that may be a determinant of CICT.

**Specific Aim 2: Quantify level of CICT in 34 inbred strains of mice (listed below) following treatment with CEM and E/C.** Pilot studies will be performed in a subset of strains to identify doses for use in the study of all 34 strains. Select biomarkers will be used to quantify cardiac toxicity.

**Specific Aim 3: Identify QTL that confer differential susceptibility to CICT.**

***Significance and Expected Outcome***

We anticipate the identification of genetic factors that confer differential CICT. It is anticipated that the genetic risk factors (orthologous genes or molecular pathways) identified in mice will influence CICT in humans and, therefore, the results will allow for a better estimation of subgroup risk.

***Current Activities***

Results are currently being developed for detecting cardiotoxicity by histopathology, ECG monitoring, serum biomarkers, and heart gene transcript analysis. Background cardiac phenotypes in multiple mouse strains are being characterized, and critical genes/pathways in the cardiotoxic process are being identified. Recently we reported that troponin serum biomarkers are more sensitive than histopathologic endpoints for detecting environmental cardiotoxicity. We are currently working to incorporate these serum biomarkers in our routine screens for hazard identification.

We have found cardiac phenotypic variation in three strains of male mice (C3H/HeJ, C57BL/6J, and B6C3F1/J) to identify candidate genes for heritable cardiomyopathy. Histopathlogic analysis identified a low-grade background cardiomyopathy in male C3H/HeJ mice (age 9-10 weeks) but not in male C57BL/6J or B6C3F1/J mice. The C3H/HeJ mouse had an increased heart rate, a shorter RR interval, and an increased QT interval in comparison to the hybrid B6C3F1/J and C57BL/6J male mice. In general, the C3H/HeJ phenotype was recessive to the C57BL/6J based upon the B6C3F1/J hybrid phenotype with possible epistatic interactions in some heritable traits. Heart gene transcript patterns segregated the three mouse strains. A significant differential expression of genes associated with cardiac disease and metabolic stress was observed. Gene Set Enrichment Analysis (GSEA) revealed that C3H/HeJ male mice manifest gene expression patterns are similar to those observed in the fetal heart. In common are a down-regulation of fatty acid oxidation and an increased expression of myosin proteins indicative of an adaptive hypertrophy, including the fetal form of the myosin heavy chain, *Myh7*. A network-based analysis indicated that the AMP kinase signaling cascade may have a critical role in driving the differential expression observed between these strains.

To identify candidate genes that may play a role in the differential manifestation of the heritable cardiomyopathy phenotype, we combined publically available expression QTL data from a BxH (C57BL/6J X C3H/HeJ) F2 cross, previously identified cardiac necrosis QTL, and a cardiac function gene list compiled using the multiple gene-centric literature mining tools. This approach identified *Prkaa2*, *Calr3,* and *Il15* as candidate genes for cardiomyopathy in C3H/HeJ mice. However, no differences in the protein coding sequences were observed between these inbred strains for these candidate cardiomyopathy susceptibility genes, hence it is hypothesized that polymorphisms in the regulatory regions of these genes cause alterations in expression that confer increased susceptibility in male C3H/HeJ mice.

***Future Plans***

Our future plans include functional validation of candidate genes through transgenic/knockout or knockin target sequence for studies in mice. This effort will take place through collaboration with intra- and extramural scientists.

Mouse strains:

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| 1 | 129S1/SvImJ | 14 | PWK/PhJ | 27 | NON/LtJ |
| 2 | A/J | 15 | WSB/EiJ | 28 | C57BL/10J |
| 3 | AKR/J | 16 | C57BL/6 | 29 | C57BLKS/J |
| 4 | BALB/cByJ | 17 | NZO/HiLtJ | 30 | C57BR/cdJ |
| 5 | C3H/HeJ | 18 | B6C3F1 | 31 | C57L/J |
| 6 | DBA/2J | 19 | PWK/PhJ | 32 | LP/J |
| 7 | FVB/NJ | 20 | CBA/J | 33 | P/J |
| 8 | NOD.B10/LtJ | 21 | MRL/J | 34 | SM/J |
| 9 | BTBRT+tf/J | 22 | PL/J | | |
| 10 | KK/HIJ | 23 | BUB/BnJ | | |
| 11 | NZW/LacJ | 24 | RIIIS/J | | PWK/PhJ replaces PWD/PhJ due to introgression. |
| 12 | CAST/EiJ | 25 | SJL/J | | |
| 13 | MOLF/EiJ | 26 | SWR/J | | |

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